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Research Article

Determination of Acceptable Residual Limit by Using Newly Developed and Validated RP-HPLC Method for Citalopram Hydrobromide Residues that Swabbed from Surfaces of Pharmaceutical Manufacturing Equipment

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ABSTRACT
In this study, easy and robust RP-HPLC method was developed and validated to determine acceptable residual limit of citalopram hydrobromide for cleaning procedures. The United States, Food and Drug Administration has mentioned that the acceptable limit for residues present in the equipment during processing or manufacturing should be based on logical criteria. In present study, acceptable residual limit for cleaning procedure of citalopram hydrobromide was calculated by using three approaches including therapeutic dose method, 10 ppm criteria and visual limit of inspection. The value by therapeutic dose approach was less among three calculated values hence, selected as acceptable residual limit (10.66 µg/25 cm^2) for analytical method development and validation for the detection of this drug residues on the surfaces of manufacturing equipment. A RP-HPLC assay method was developed using C18 column and mobile phase containing mixture of methanol and ammonium acetate buffer (pH4.6) (70:30) with flow rate of 1.6 ml/min. System suitability parameters like theoretical plates and tailing factor were calculated for the validation of the developed method as per ICH Q2B guidelines. After analyzing recovery study data, it was found that stainless steel plate has minimum recovery (88.87%) and pre-analyzed tablet solution has maximum recovery (99.29%).

Keywords: Acceptable residual limit, therapeutic dose method, visual limit of inspection, citalopram hydrobromide and ICH Q2B.

INTRODUCTION

Many pharmaceutical products have been recalled from the market worldwide due to cross-contamination with pharmaceuticals and other chemicals. In pharmaceutical industry as well as in food industry, the removal of possible residues from the surfaces of production equipment to avoid cross-contamination is becoming a very important requirement. In most of the production lines the same equipment is used for the processing of different types of products. Thus, effective cleaning procedures are necessary steps in order to avoid contamination of subsequent products. As per new guidelines from regulatory authorities, equipment must be cleaned before and after processing, also all cleaning steps must be documented. The analytical methods used to analyze residues must be robust, sensitive, rapid and selective. The very important factors for measurements of effectiveness of reliable cleaning are standardized sampling procedure and consistent recovery. For experimental work, a helpful document for cleaning validation is US FDA's guidance for determining residue limits. [1] The proposal made in this document is that the equipment must be visually clean, with a maximum carryover limit of 10 ppm to the subsequent product as a general rule. The tolerance limit for an active pharmaceutical agent is 1/1000 of the minimum daily dose from previous product in the maximum daily dose of the subsequent product (Fourman, Mullen, 1998).

The cGMP in the United State, Europe and other countries of the world has provided the pharmaceutical industry with general guidelines for cleaning requirements. For example in USFDA 1963 cGMP regulation (Part 133.4) states *that "Equipment shall be maintained in a clean and orderly manner….."*. A very similar section *on* equipment cleaning (211.67) was included in 1978 cGMP regulation, it states "*Equipment and utensils shall be cleaned maintained and sanitized at appropriate intervals to prevent malfunctioning and contamination that would alter the safety, identity, strength, quality and purity of the drug product beyond the official or other established requirements"* (Harder, 1984; Asgharian *et al.*, 2014).

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Cleaning should also address in the PIC (Pharmaceutical Inspection Convention) recommendation on cleaning validation.

Citalopram hydrobromide (Figure 1) is a selective serotonin reuptake inhibitor (SSRI) with a chemical structure unrelated to other SSRIs or of tricyclic, tetracyclic or other available antidepressant agents. It is a racemic bicyclic phthalein derivative and commercially available as tablet (10 to 60 mg strengths) or as an oral solution. Due to sparingly solubility in water and low dose profile it is necessary to prove that the equipment train and production area should be clean prior to development of next product, as per cGMP (good manufacturing practices) guidelines (Agalloco, 1992).

Citalopram hydrobromide tablets 20 mg were formulated by utilizing common facility in the Rajiv Gandhi Prodhyogiki Visswavidyalaya, Bhopal, India where API could be a possible cross contaminant. Hence the present study was carried out to validate the cleaning activity from both regulatory and quality prospective (Shah *et al.*,2014).

MATERIAL AND METHODS

Cetalopram hydrobromide was provided by Ranbaxy laboratories Ltd., India, as a gift sample. Methanol (HPLC grade), acetonitrile (HPLC grade) and hydrochloric acid were purchased from Merk Ltd., India. Formic acid, glacial acetic acid, ammonium acetate and sodium acetate were obtained from CDH chemical Ltd., India. Cotton swabs were purchased from Himedia bioscience, India.

Selection of diluent for sample preparation

On the basis of solubility studies of citalopram hydrobromide in different solvents, methanol, ethanol and acetonitrile were selected for extraction of this drug from cotton swab.

Absorption maxima (λ_{max}), melting point and pka value of drug

To select the absorption maxima of citalopram hydrobromide a solvent system of water and methanol (1:1) was selected. The solution was scanned by using UV- spectrophotometer and absorption maxima was observed.

Melting point and pKa value for the drug were also determined.

Establishment of cleaning level acceptance criteria for citalopram hydrobromide

There are several approaches used for the establishment of acceptable residual limit (ARL) calculation. In our experimental work ARL is calculated and compared with different approaches and the minimum value of ARL was selected.

Calculation of total carryover limits based on therapeutic or medical dosages

The smallest ARL or the lowest allowable residue level based on pharmacological activity is achieved by using the smallest dosage of the current product and the smallest batch size manufactured using the equipment train. The formula used for the calculation of the ARL value is given below (Fourman *et al.*, 2006; LeBlanc, 1998).

ARL= (STD×SBS×SF×M)/ (MDD×SSA)

Where

ARL: Acceptable residual limit

STD: Smallest therapeutic dose of product A (mg/unit dose), STD for citalopram hydrobromide is 10 mg

SBS: Smallest batch size of any subsequent product to be manufactured in the same equipment train

Smallest batch size= Minimum working capacity in gm (A)/ Weight of tablet (B)

SF: Safety factor i.e. 1/1000 or 0.001

MDD: Maximum daily dose of the product to be manufactured in the same equipment train. In this experiment MDD of Product B was 5 mg

M: Surface area/swab (25 cm^2)

SSA: Shared equipment surface area

Shared surface area of the tablet compression machine (RIMEK MINI-II MT 12STN) Covering hopper, lid of hopper, turret, feed frame and discharge chute was calculated 3112.41 cm^2.

Limit of calculation based on 10 ppm criteria (adulteration limit)

It is stated that not more than 10 ppm of the product will appear in the next batch of product. ARL was calculated on the bases of 10 ppm criteria according to below formula.

ARL= (10×MBS×M)/ SSA

Where,

ARL: Acceptable residual limit

MBS: Minimum batch size in Kg of any subsequent product to be manufactured in the same equipment train. (Product B)

M: Surface area/swab (25 cm^2)

SSA: Shared equipment surface area

Minimum batch size 1.25 L (25% capacity of hopper) × 425 g/L (avg. bulk density of subsequent material)

Limit of calculation on the bases of visual inspection

Visually equipment surfaces area must appear clean with no traces of product or any extraneous matter. The VLOD (visual limit of detection) was determined by spiking five 5×5 cm^2, 316 stainless steel plates with known amount of drug.

Development of HPLC procedure

Preparation of reagents

Preparation of acetate buffer (pH 4.6)

About 385.0 mg of ammonium acetate was weighed and dissolved in 250 ml of Millipore water. The pH of buffer was adjusted to 4.6 with the help of glacial acetic acid.

Preparation of stock solution and dilutions

Accurately weighed 10 mg of citalopram hydrobromide working standard was transferred in to a 10 ml volumetric flask. It was dissolved by adding sufficient amount of methanol (HPLC grade) and volume was made up with methanol to produce a solution of 1000 µg/ml of citalopram hydrobromide. Further dilutions made to produce a solution of 10 ppm.

Selection of HPLC parameters

Reverse phase C_{18} column was selected for separation because the drug was polar in nature. According to

literature reports and absorption maxima of drug observed by UV scans, the λmax of drug was selected at 239 nm.

Selection of mobile phase

Mobile phase was selected by hit and trial method on the basis of solubility behavior and pKa value of the drug. Considering the system suitability parameters viz retention time, tailing factor, number of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was the mixture of methanol and acetate buffer (pH 4.6) in the ratio of 70: 30(methanol: acetate buffer pH4.6). The mobile phase was filtered through 0.45 µm nylon filter paper to remove particulate matter and then degassed by sonication.

Selection of column temperature, flow rate of mobile phase, and selection of swabs

Ambient column temperature and isocratic flow with flow rate of 1.6 ml/min flow rate was selected. At lower flow rate RT was longer and peak was broad. Recovery study was performed for two brands, sterile cotton swabs in HDPE tubes with polypropylene stick and Cutisorb^{TM} absorbent cotton pad in an absorbent cotton overwrap for the selection of swabs.

Final optimized method

Preparation of mobile phase A

About 385 mg of ammonium acetate was weighed and dissolved in 250 ml of Millipore water. The pH of buffer was adjusted to 4.6 with the help of glacial acetic acid. Then the buffer solution was filtered through 0.45 µm nylon filter and subjected to sonication for 10 min.

Mobile phase B

About 1000 ml of methanol was transferred to 1000 ml mobile phase bottle and kept at room temperature. Then it was filtered through 0.45 µm nylon filter and subjected to sonication for 10 min.

Preparation of diluent

Methanol was selected as diluent. About 500 ml of methanol was transferred in 500 ml of flask and it was filtered through 0.45 µm nylon filter.

Preparation of system suitability solution

Accurately weighed about 10 mg of citalopram hydrobromide and transferred to a 10 ml volumetric flask, sufficient volume of methanol was added to dissolved it and volume was made up to 10 ml (stock A; 1000 µg/ml), 1.0 ml of stock A was taken into 10 ml volumetric flask and further diluted up to 10 ml with methanol (stock B; 100 µg/ml). Aliquots of stock B were further diluted up to 10 ml to get concentration of 10, 20, 30, 40, 50 µg/ml. The System precision was evaluated by performing six replicate injections of the standard citalopram hydrobromide solution 30 µg/ml.

Validation of developed method as per ICH Q2B guideline (Ermer, Miller, 2006)

Linearity and calibration curve

In order to establish the linearity of analytical method, a series of dilutions ranging from 1-50 µg/ml was prepared. All the solutions were filtered through 0.45 µm nylon filter. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20 µl). Chromatograms were recorded & calibration curve was plotted between the mean peak area vs. respective concentration and regression equation was obtained (Figure 2).

Linearity range

In order to achieve working concentration range, linearity was observed in the range of 1 to 50 µg/ml at RT 1.6. Also, samples of different concentrations of drug 1, 5, 10, 20, 30, 40, 50 µg/ml were prepared and the chromatograms was recorded. Peak area of chromatograms was recorded as well as calculated the response ratios.

Specificity

Specificity of the HPLC method is demonstrated by the separation of the analyte from other potential components such as impurities, degradants, or excipients. Equivalent weight of 10 µg drug was weighed from a tablet (CELEXA by Cyril Pharmaceuticals) and a 20 µg/ml solution was prepared. This was analyzed using proposed method.

Accuracy

To test accuracy recovery study was performed. A definite concentration of standard drug was added to a pre-analyzed sample of citalopram hydrobromide tablet (CELEXA), and then its recovery study was performed. This was repeated with different concentrations of standard drug. It was repeated three times to emphasize validation.

Recovery study of drug from swabs, spiked SS plates and Teflon plates

Recovery studies were performed on spiked swabs, stainless steel plates and Teflon plates with a predefined 25 cm^2 surface area. A 5-ml volume of different concentrations of 1, 5, 20, 50 and 100 µg/ml of citalopram hydrobromide were spiked onto swabs, stainless steel plates and Teflon plates with five sets of concentrations of five plates each, and were allowed to evaporate. The head of the absorbent swabs was saturated with methanol. The total surface of the plates was successively wiped initially in a horizontal and then in a vertical fashion, starting from the outside towards the center, with swabs moistened with the appropriate solvent. The head of the swab(s) was placed into a 10 ml volumetric flask containing 5.0 ml of the solvent (in which the swab was soaked). Then 5 ml of water was added to each volumetric flask. These volumetric flasks were capped and sonicated for 15 min and filtered through 0.45 µm nylon filter. The extract was finally transferred into injection port.

Precision

The precision data for the method were obtained by repeated injection of different concentration of the drug.

Repeatability

Repeatability with drug solution (Intra-day repeatability)

The precision (Intra-day repeatability) was established by giving three replicates of three concentrations (10, 20 and 30 µg/ml). In intraday precision, all the replicates were injected same day and statistical analysis was carried out.

Repeatability on SS plates

Assay repeatability was studied for 30 µg level by spiking it on five separate plates of stainless steel.

Inter-day precision

Day to day precision (inter-day) was carried out by giving three concentrations (10, 20 and 30 µg/ml) with three replicates each. In intra-day precision, all the linearity concentrations were prepared and chromatograms were recorded for three days.

Analyst to analyst

Analyst to analyst precision was performed by 2 analysts using three concentrations (10, 20 and 30 µg/ml) with three replicates each. Mean, SD and %RSD was calculated for all the concentrations.

Robustness

Robustness of method was calculated by making small, deliberate changes in the condition like pH variation (4.6±0.2), mobile phase variation (±2%), flow rate variation (±0.2 ml/min) with 3 different concentrations (10, 20 and 30 µg/ml) and 3 replicates each. The % RSD was also calculated for each condition.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined experimentally by using signal to noise (S/N) ratio method.

Stability of solution

A set of standard solutions ranging from 10- 50 µg/ml were prepared and placed on bench-top at ambient conditions away from sunlight. After Day-1, Day-2 and Day-3 the stability of solutions was analyzed and compared to freshly prepared solutions of drug. We checked the stability of the analytical solutions for 48 hours.

Stability study for SS plate, Teflon plate, dry swab, wet swab

Three sets of '180-Grit' stainless steel and Teflon plates were spiked with 20, 30, 40 µg of citalopram hydrobromide (with methanol as a diluent), and set aside undisturbed for four days. Another set of SS plates spiked with same concentration were swabbed immediately, swabs were transferred in (High-density polyethylene) HDPE bottles, caped securely and allowed to stand undisturbed. This set was labeled 'Dry swabs'. One more set of stainless steel plates spiked with same concentration were swabbed immediately, placed in HDPE bottles containing solvent and capped securely. This set was labeled as 'Wet swabs'

RESULT AND DISCUSSION

Selection of diluent for sample preparation

With methanol, the drug was showing maximum recovery hence methanol was selected as a solvent and as a diluent for extraction (Table I).

Absorption maxima (λ_{max}), melting point and pKa value of drug

A characteristic spectrum of drug was observed with absorption maxima at 239 nm. Melting point of drug was found to be 186-188 °C, which was well according to the reported literature (185 -188 °C) and pKa value of drug was found to be 9.5.

Calculation of acceptable residual limit

Acceptable residual limit was calculated on the basis of medical or therapeutic dose, 10ppm criteria and visual limit of detection (VOLD), and their corresponding values found to be 10.66, 46.65 and 45.00 µg per 25 cm^2, respectively. Since ARL value (10.66 µg/25 cm^2) was the lowest value among all values, therefore the method based on therapeutic or medical dosage was selected as ARL value.

Development of assay method

In order to select the mobile phase for the system, first of all solubility studies were conducted and it was found that citalopram hydrobromide was soluble in the all the organic solvent and insoluble in water (solubility detected by visual inspection). According to literature and result of UV scan, 239 nm was selected as absorption maxima as shown in Figure 3.

On the basis of solubility of drug, many trials were conducted with combination of solvent to select the mobile phase for system on C_{18} column (drug was polar). After selecting all the variables; system suitability parameters like retention time, tailing factor, no. of theoretical plates and HETP were found to be most suitable for analysis of drug in mobile phase containing methanol: ammonium acetate (70:30) at flow rate of 1.6 by using photo diode array detector. Ambient temperature was found to be suitable for the system (Figure 4).

Maximum recovery of spiked drug was in methanol; therefore, methanol was selected as diluent (Table I). After recovery study from different types of swabs, it was found that HiMedia PW003 Swabs has 89.33% recovery which was greater than of CutiSorb™ (75.89%) (Table II). Hence sterile cotton swabs in HDPE tubes with polypropylene stick (HiMedia PW003) were selected (Table III and Table IV).

Validation of developed assay method

Validation of developed method was carried out by determining various parameters according to ICH Q2B guidelines.

Linearity

Citalopram hydrobromide drug shows linearity in range between 1-50 µg/ml.

Specificity

After analyzing the tablet sample by a previously developed method it was observed that there was no interference observed in chromatographic run in presence of excipients.

Accuracy

Recovery study of the developed method was performed on pre-analyzed tablet sample solution, '180-Grit' stainless steel plates, Teflon plates and cotton swabs. Accuracy was expressed as percentage of standard dug recovered from sample matrix with corresponding %RSD. The range of recovery study was selected according predetermined ARL value. The ARL value was 10.66 µg/25 cm^2; hence range for recovery study was selected as 5-100 µg/25 cm^2.

Precision

Data for repeatability and intermediate precision studies at three different concentrations within linearity range are described in the Table V.

The %RSD values for both repeatability and intermediate precision were found within limit, hence the method developed was precise.

Robustness

To check the robustness of the method, deliberate changes were done in the developed procedure and data were analyzed as shown in Table VI.

Limit of detection (LOD) and limit of quantitation (LOQ)

Limit of detection (LOD) can be defined as the concentration that yields a signal to noise ratio of 3 that was found to be 100 ng/ml. The limit of quantitation (LOQ) was calculated as the lowest concentration that could be measured with a signal to noise ratio 10 and was found to be 200 ng/ml. The smallest level at which the recovery of citalopram hydrobromide was determined; 5 µg/ml, it was considered as lowest limit of quantitation for residual determination. This LOQ level was significantly lower than the calculated ARL value (10.66 µg/25 cm^2).

Stability study of Citalopram hydrobromide on stainless steel, Teflon surface and in swabs

Stability study for Citalopram hydrobromide was studied on dry plates, wet swabs, dry swabs and Teflon plates for four days. The samples were analyzed at initial day, day-1, day-2 and day-3.

The dry plate stability indicated the length of time during which drug remains in contact with equipment surface prior to initiation of the swabbing process.

The dry swab and wet swab stability indicated the length of time from the swabbing of drug to the starting of analysis process, and this test indicated the stability of drug in contact with swab surface.

The results indicated that the drug is stable up to two days on all the surfaces. Hence it recommended that samples should be immediately swabbed and immediately analyzed (Table VII).

SUMMARY AND CONCLUSION

Citalopram hydrobromide is considered as a potent drug due to its low dose profile. So before manufacturing the next product in the same equipment train, its removal from the manufacturing equipment train is necessary. From this research, it can be concluded that the proposed HPLC method for Citalopram Hydrobromide is easy, accurate, reproducible, robust and sensitive. The results showed that this method is suitable for quantitative determination of residues of citalopram hydrobromide in production area equipment, below the calculated limit of contamination. After development of analytical method, the further task is to develop and validate a cleaning procedure for the removal of citalopram hydrobromide from the manufacturing equipment train. The reproducibility of the validated analytical method and cleaning procedure can be checked by formulating the tablet batches of another drug in the same equipment train i.e. equipment used during cleaning procedure validation.

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